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**Research Note**

# Effects of Polyphosphate Additives on the pH of Processed Chicken Exudates and the Survival of *Campylobacter*†

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## ABSTRACT

*Campylobacter* spp. are nutritionally fastidious organisms that are sensitive to normal atmospheric oxygen levels and lack homologues of common cold shock genes. At first glance, these bacteria seem ill equipped to persist within food products under processing and storage conditions; however, they survive in numbers sufficient to cause the largest number of foodborne bacterial disease annually. A mechanism proposed to play a role in *Campylobacter* survival is the addition of polyphosphate-containing marinades during poultry processing. *Campylobacter jejuni* and *Campylobacter coli* strains incubated in chicken exudates collected from poultry treated with a marinade demonstrated considerable survival advantages (1 to 4 log CFU/ml) over the same strains incubated in chicken exudate from untreated birds. Polyphosphates, which constitute a large portion of the commercial poultry marinades, were shown to account for a majority of the observed influence of the marinades on *Campylobacter* survival. When six different food grade polyphosphates (disodium pyrophosphate, tetrasodium pyrophosphate, pentasodium triphosphate, sodium polyphosphate, monosodium phosphate, and trisodium phosphate) were utilized to compare the survival of *Campylobacter* strains in chicken exudate, significant differences were observed with regard to *Campylobacter* survival between the different polyphosphates. It was then determined that the addition of polyphosphates to chicken exudate increased the pH of the exudate, with the more sodiated polyphosphates increasing the pH to a greater degree than the less sodiated polyphosphates. It was confirmed that the change in pH mediated by polyphosphates is responsible for the observed increases in *Campylobacter* survival.

*Campylobacter* presents a serious risk to food safety, accounting for the greatest number of foodborne gastrointestinal bacterial infections annually in the developed world *(1, 6, 12)*. The main source for introducing *Campylobacter* into the food supply is poultry products *(9, 20)*. However, *Campylobacter* spp. are known to be nutritionally fastidious and require elevated temperatures for growth (37 to 42°C) and a microaerobic atmosphere for survival *(12)*. Therefore, because of these traits, *Campylobacter* spp. do not initially appear to be excellent candidates for maintaining bacterial numbers in food products sufficient for causing the considerable amount of disease for which they are responsible.

In an effort to understand how *Campylobacter* is able to function as a successful food pathogen, previous research from our laboratory described a food practice that has the potential for enhancing the ability of *Campylobacter* to persist on poultry products through processing and cold storage *(8)*. Polyphosphate-containing marinades added to chicken products were shown to enhance the numbers of *Campylobacter jejuni* and *C. coli* strains surviving in the exudates derived from the marinated chickens. Polyphosphates are a group of food grade compounds generally regarded as safe that can be added to poultry and other meat products during processing. Polyphosphates are used to increase the ability of the meat product to hold water, to enhance taste, to prevent product from drying out during cooking, and in some cases as antimicrobial agents *(3, 5, 7, 17, 21–24)*.

Exudates from poultry treated with polyphosphate-containing marinade or without marinade have been observed to possess different pH values, suggesting that change in pH of the environment may be responsible for the enhanced numbers of *Campylobacter* observed in the polyphosphate-containing exudates *(8)*. The research presented in this article investigates the effects that pH changes independent of polyphosphate addition have upon *Campylobacter* numbers in poultry exudates. Therefore, the goal of this work was to determine if pH changes caused by polyphosphates play a role in the increased *Campylobacter* survival described in previous research *(8)*. Additionally, the pH differences in exudates mediated by different polyphosphates and their subsequent effects on *Campylobacter* survival were investigated.

## MATERIALS AND METHODS

**Bacterial strains.** *C. coli* strains RM1403, RM4764, and RM1529 and *C. jejuni* strains RM1221, RM1188, and RM3194

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† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

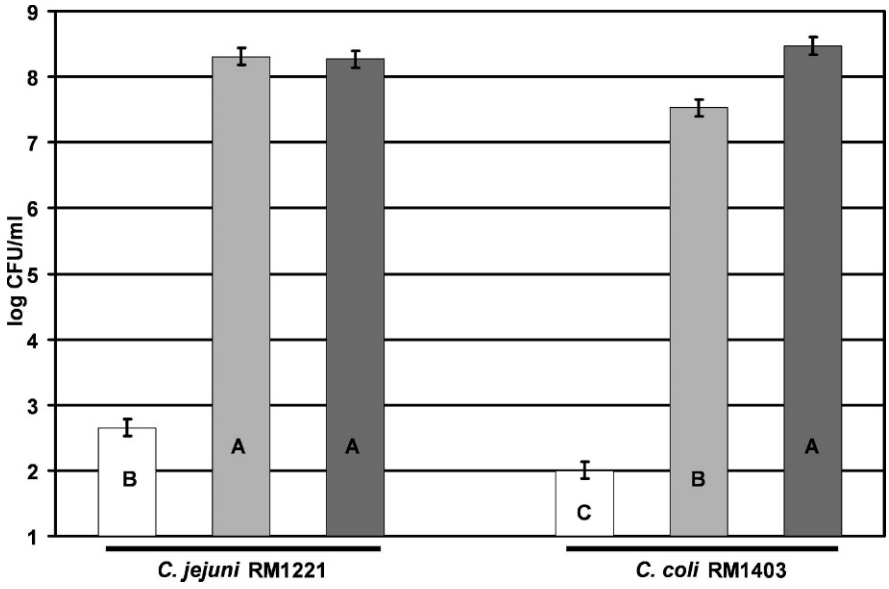


FIGURE 1. *Populations (CFU per milliliter) of* C. jejuni *strain RM1221 and* C. coli *strain RM1403 after incubation at 42°C microaerobically for 24 h in exudate alone (pH 5.76) (□); in exudate plus NaOH (pH 6.42) (▨); and in exudate plus polyphosphate-containing marinade (pH 6.42) (■). Samples labeled with different letters were considered to differ with statistical significance (*$P < 0.05$*) from one another with regard to the number of culturable* Campylobacter *cells present in each.*

were kindly provided by Dr. Robert Mandrell (U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Albany, CA). Strains were cultured from frozen stocks (−80°C) directly onto Brucella (BD, Sparks, MD) agar plates (1.5% agarose). Overnight cultures were grown in Brucella (BD) broth and incubated in a microaerobic chamber (MACS-VA, Don Whitley, UK) (5% $O_2$, 10% $CO_2$, 85% $N_2$) at 42°C.

**Chicken exudate collection and preparation.** Prior to packaging, the exudates from both marinated and nonmarinated chicken products were collected at the processing plant of a commercial poultry producer. Exudates from either marinated or nonmarinated chicken products were collected into plastic containers directly off the processing line and from multiple chicken carcasses. The nonmarinated chicken products were chicken carcasses that had been prepared for consumer sale and cleaned and chilled with water only. Marinated chicken products were chicken carcasses prepared for consumer sale that had been cleaned and chilled with water and then treated with a marinade consisting of a mixture of polyphosphates (final concentration, 0.5%) and dehydrated chicken broth powder (final concentration, 0.06%) dissolved in a water base and applied within a vacuum tumbler apparatus. Initially, both forms of the collected exudates were filtered with cheese cloth to remove large pieces of chicken meat and fat. The exudates were then aliquoted into conveniently sized containers and frozen at −80°C. Next, the frozen aliquots were irradiated to sterility using a self-contained 137Cs gamma irradiator (Lockheed Georgia Company, Marietta, GA) at a dose rate of 0.086 kGy/min *(19)*. The temperature during irradiation was maintained at 0°C through the introduction of gas phase liquid nitrogen directly into the top of the sample chamber.

**Conditions for survival experiments.** Survival experiments in this article were performed in a manner similar to experiments described in a previous research article *(8)*. Briefly, 5 μl (~$10^9$ CFU/ml for *C. jejuni* or ~3 × $10^8$ CFU/ml for *C. coli*) from overnight Brucella broth cultures of the appropriate *Campylobacter* strains was used to inoculate 5 ml of the treated or untreated chicken exudates investigated in these experiments. Next, cultures were either incubated at 42°C in a microaerobic chamber (described above) or at 4°C in a normal atmosphere in a refrigerated incubator (Innova 4230, New Brunswick Scientific, Edison, NJ). Regardless of conditions or media, all *Campylobacter* cultures were incubated statically to replicate commercial conditions as closely as possible. Upon the completion of the incubation periods (24 h for microaerobic conditions at 42°C and 0 to 336 h for experiments in a normal atmosphere at 4°C), the viable *Campylobacter* organisms present in each culture were enumerated by using the 6 × 6 drop plate method with six replicates per dilution *(2)*. For the experiments investigating the effects of increased pH conditions, sodium hydroxide (1 M NaOH) was used to shift exudate pH from 5.76 to 6.42, and for the experiments investigating the effects of decreased pH conditions, hydrochloric acid (1 M HCl) was used to shift the pH of exudate plus marinade from 6.42 to 5.76. The six different food grade polyphosphates utilized in this research (disodium dihydrogen pyrophosphate, monosodium dihydrogen phosphate, sodium hexametaphosphate, pentasodium triphosphate, tetrasodium pyrophosphate, and trisodium phosphate) were supplied by ICL Performance Products LP (St. Louis, MO). All polyphosphates were used at a final concentration of 0.5% (wt/vol).

**Statistical analyses.** An analysis of variance was performed by using the ''Mixed'' procedure of the SAS Software System to determine the effects and interactions of species and conditions *(16)*. Mean separations were performed by using the Bonferroni least significant difference technique *(11)*. Analyses were performed separately for *C. jejuni* strains and *C. coli* strains. All statistical tests of significance were performed at $P$ values of $<0.05$.

## RESULTS

**Increased pH results in increased *Campylobacter* numbers.** When exudate collected from unmarinated chicken products was treated with NaOH shifting the pH from 5.76 to 6.42, there was a significant increase in *C. jejuni* and *C. coli* cell numbers compared with unmarinated exudate with a pH of 5.76 (Fig. 1). The resulting numbers of *C. jejuni* strain RM1221 present in the unmarinated exudate with the pH shifted to 6.42 were statistically equal to the *C. jejuni* present in the exudate from marinated chicken after 24 h of incubation at 42°C in a microaerobic environment. The numbers of *C. coli* strain RM1403 cells in the unmarinated exudate with the pH increased to 6.42 were similar but statistically less than the numbers of the same

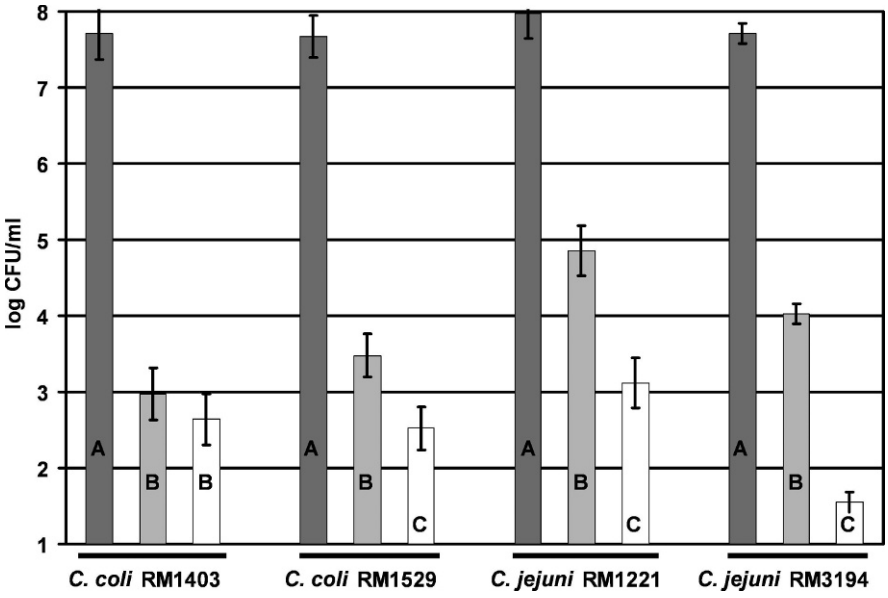


FIGURE 2. *Populations (CFU per milliliter) of* C. jejuni *strains RM1188 and RM3194 and* C. coli *strains RM1403 and RM1529 after incubation at 42°C microaerobically for 24 h in exudate alone (pH 5.76) (□), in exudate plus phosphate-containing marinade and HCl (pH 5.76) (▒), and in exudate plus polyphosphate-containing marinade (pH 6.42) (■). Samples labeled with different letters were considered to differ with statistical significance (*$P < 0.05$*) from one another with regard to the number of culturable* Campylobacter *cells present in each.*

strain in exudate plus marinade with an unadjusted pH of 6.42. Therefore, increasing the pH of the unmarinated exudate to 6.42 increased the numbers of both a *C. jejuni* strain and a *C. coli* strain by just over 5 log compared with when the bacteria were incubated in the unmarinated exudate with an unmodified pH of 5.76.

**Decreased pH results in decreased *Campylobacter* numbers.** The experiment complementary to the previous study used the exudate collected from marinated chickens with a normal pH of 6.42 and reduced the pH through the addition of HCl to 5.76, the same pH as that of exudate from unmarinated chickens. The *Campylobacter* strains incubated in the exudate plus marinade with the pH reduced by adding HCl had significantly fewer bacteria, between 3 and 5 log less, than did the same strain of *Campylobacter* incubated in exudate plus marinade with the normal pH of 6.42 (Fig. 2). When incubated in the exudate plus marinade with a reduced pH of 5.76, *C. jejuni* RM1221 and RM3194 produced bacterial numbers similar to those produced by the same strains incubated in exudate without marinade also with a pH of 5.76. All strains in the two different exudates were incubated for 24 h at 42°C in a microaerobic environment. Compared with growth in marinated exudate (pH 6.42), the two *C. coli* strains RM1403 and RM1529 also produced similarly reduced numbers when incubated in chicken exudate plus marinade with the pH lowered to 5.76 and in chicken exudate with no marinade with a normal pH of 5.76. Strain RM1403 produced statistically equal bacterial numbers under the two reduced pH conditions (Fig. 2).

**Resulting pH values of different polyphosphates added to chicken exudate to a final concentration of 0.5%.** Six different food grade polyphosphates were individually added to exudates collected from unmarinated chickens at final concentrations of 0.5%. The chicken exudate with no added polyphosphates had a pH of 5.80 (±0.07) (Table 1). Two of the polyphosphates, disodium dihydrogen pyrophosphate and monosodium dihydrogen phosphate, produced pH values in exudate less than those demonstrated in exudate alone, 5.43 (±0.04) and 5.63 (±0.07), respectively. Sodium hexametaphosphate produced a pH of 5.88 (±0.06) when added to exudate, a pH value statistically similar to that of exudate alone. Pentasodium tripolyphosphate, tetrasodium pyrophosphate, and sodium triphosphate all produced pH values when mixed with exudate statistically higher than exudate alone, 6.28 (±0.04), 6.51 (±0.10), and 7.06 (±0.04), respectively. The polyphosphates that produced pH values similar to or less than exudate alone have chemical structures with only one sodium atom per phosphate group. Conversely, when mixed with exudate, the polyphosphates that produced pH values statistically greater than those of exudate alone all have chemical structures with greater than one sodium atom per phosphate group.

***Campylobacter* survival in different polyphosphate exudate solutions.** Strains of *C. jejuni* and *C. coli* incubated in six different polyphosphate (concentration, 0.5% by weight) chicken exudate solutions produced significantly different bacterial numbers after incubation at 42°C

TABLE 1. *Commonly used polyphosphates in poultry processing, pHs resulting from a 0.5% (wt/vol) solution of the polyphosphates in chicken exudate, and ratios of sodium atoms to phosphate groups*

| Polyphosphate | pH (±SD) of exudate plus 0.5% polyphosphate | Ratio of sodium to phosphate |
|---|---|---|
| No polyphosphate (control) | 5.80 (±0.07) | NA[a] |
| Disodium dihydrogen pyrophosphate | 5.43 (±0.04) | 1:1 |
| Monosodium dihydrogen phosphate | 5.63 (±0.07) | 1:1 |
| Sodium hexametaphosphate | 5.88 (±0.06) | 1:1[b] |
| Pentasodium triphosphate | 6.28 (±0.04) | 5:3 |
| Tetrasodium pyrophosphate | 6.51 (±0.10) | 2:1 |
| Trisodium phosphate | 7.06 (±0.04) | 3:1 |

[a] NA, not applicable.
[b] $Na_{(n+2)}P_nO_{(3n+1)}$; average $n = 21$.

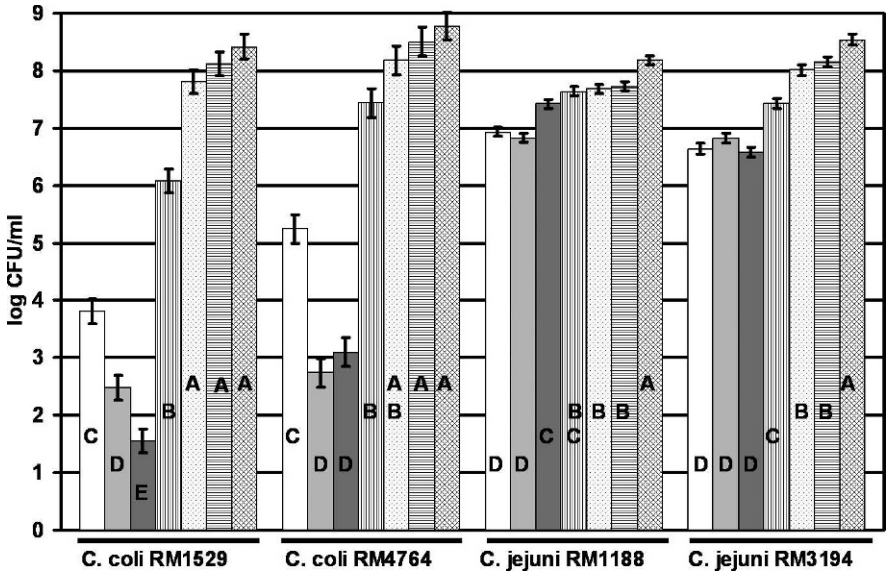


FIGURE 3. *Populations (CFU per milliliter) of* C. jejuni *strains RM1188 and RM3194 and* C. coli *strains RM4764 and RM1529 after incubation at 42°C microaerobically for 24 h in exudate plus no polyphosphate (control) (□), plus disodium dihydrogen pyrophosphate (▤), plus monosodium dihydrogen phosphate (■), plus sodium hexametaphosphate (▥), plus pentasodium triphosphate (⬚), plus tetrasodium pyrophosphate (▤), and plus trisodium phosphate (▨). Samples labeled with different letters were considered to differ with statistical significance (*$P < 0.05$*) from one another with regard to the number of culturable* Campylobacter *cells present in each.*

microaerobically for 24 h compared with exudate alone or between the different polyphosphates (Fig. 3). The numbers of surviving *Campylobacter* increased from one polyphosphate exudate to the next as the pH produced by the polyphosphate exudate solutions also increased. The polyphosphate trisodium phosphate produced the greatest pH change compared with the exudate alone and the other polyphosphates. With *C. jejuni,* both RM1188 and RM3194 numbers were increased by between 1 and 2 log when comparing the exudate alone and the exudate plus 0.5% trisodium phosphate. The numbers of *C. coli* cells of both strains RM1529 and RM4764 in exudate plus trisodium phosphate increased between 3 and 5 log over the numbers in exudate alone. Additionally, the polyphosphates disodium dihydrogen pyrophosphate and monosodium dihydrogen phosphate, which produced pH values lower than exudate alone, also resulted in *C. coli* numbers significantly less than the level present in exudate without added polyphosphates.

***Campylobacter* survival in different polyphosphate exudate solutions under cold storage conditions.** The previous experiments were repeated in more relevant food storage conditions (4°C in a normal atmosphere) with regular sampling over a 2-week period. The results for *C. jejuni* RM1221 incubated in exudate plus the different polyphosphates were similar to the results in the previous experiment (Fig. 3), except that it took a longer time before significant differences in *Campylobacter* numbers in the different polyphosphates began to develop (Fig. 4A). Between 168 h and 336 h (1 week to 2 weeks), significant differences were observed between the numbers of bacteria surviving in the various polyphosphate cultures, with the polyphosphates producing the larger increases in pH values also producing the most significant advantage in *C. jejuni* strain RM1221 survival.

Similar results were observed using *C. coli* strain RM1403 (Fig. 4B). At the 168-h (1-week) sample time point, we again observed significant differences in bacterial numbers with the greatest bacterial survival corresponding to the polyphosphates producing the greatest increase in exudate pH; and these differences remained significant through 336 h (second week).

## DISCUSSION

The research presented in this article in combination with previous work has implicated polyphosphates added to poultry during processing as a possible risk to food safety *(8).* Polyphosphates were shown to increase the pH of chicken exudate and to enhance the survival of *C. jejuni* and *C. coli* strains incubated in these exudates under conditions that simulate food storage (4°C, normal atmosphere). The observed difference in *Campylobacter* survival due to the presence of polyphosphates ranged from 1 to 4 log CFU/ml, a difference that is significant given previous research showing that a decrease of just 2 log CFU/ml in poultry products was predicted to reduce the risk of campylobacteriosis 30-fold *(15).*

The poultry industry and other meat processors rely on the use of polyphosphates for the characteristics they impart to the meat. Polyphosphates enhance water retention in poultry products, influence color and taste, and help prevent drying of the meat product during cooking *(17, 21–24).* However, as was demonstrated by the presented data, different polyphosphates also shift the pH of the poultry product environment by greater or lesser amounts. We observed that greater pH shifts more closely approaching the pH values preferred by *Campylobacter* spp. (pH 6.5 to 7.5) resulted in greater survival of the bacteria than with smaller pH shifts *(4, 18).* Therefore, from a food safety perspective it appears preferable that the poultry industry use polyphosphates that shift pH away from an acidic pH by the smallest amounts (disodium dihydrogen pyrophosphate, monosodium dihydrogen phosphate, and sodium hexametaphosphate) rather than those that cause larger shifts in pH (pentasodium tripolyphosphate, tetrasodium pyrophosphate, and trisodium phosphate).

This work has demonstrated that polyphosphate-mediated changes in pH are responsible for the observed

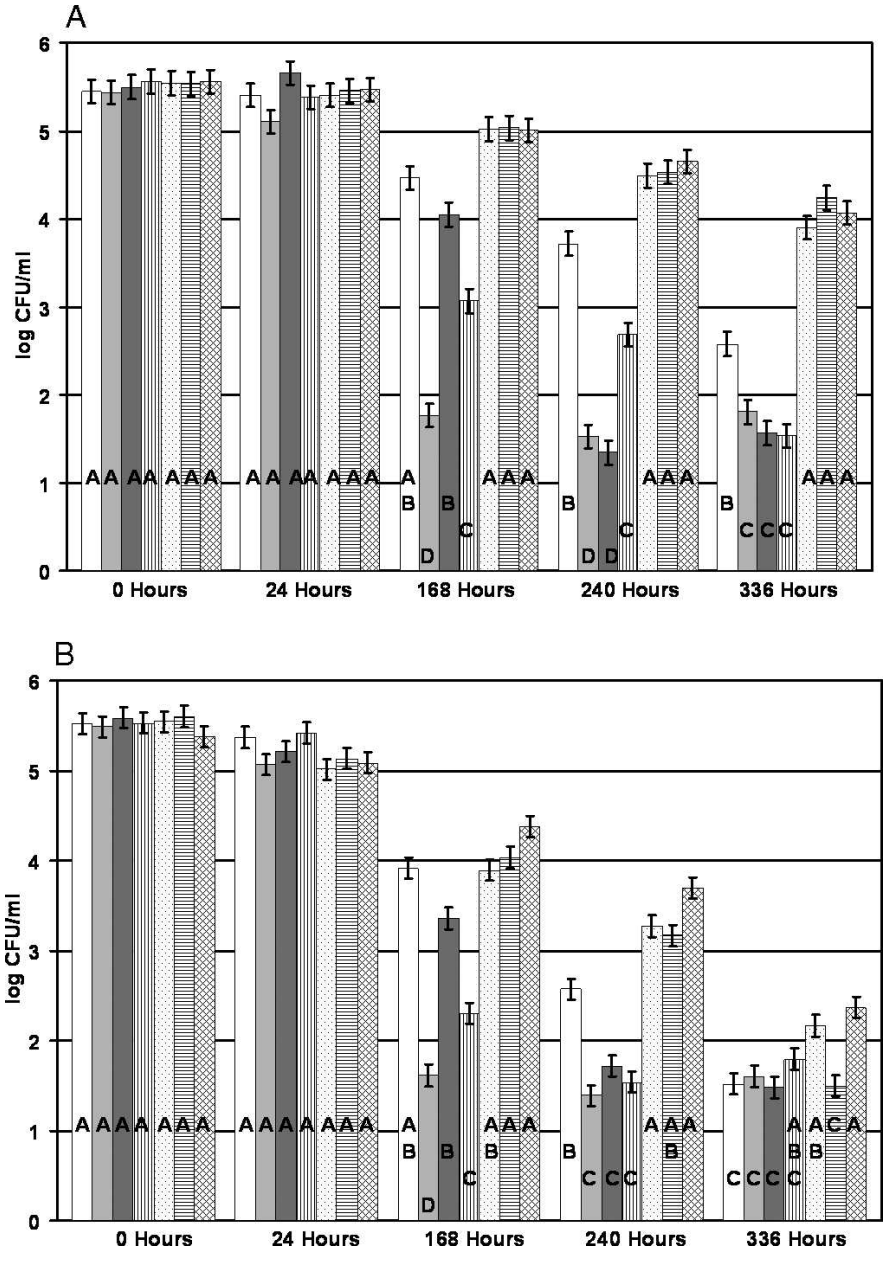


FIGURE 4. *Populations (CFU per milliliter) of* C. jejuni *strain RM1221 (A) and* C. coli *strain RM1403 (B) incubated for 2 weeks at 4°C in a normal atmosphere in exudate plus no polyphosphate (control) (□), plus disodium dihydrogen pyrophosphate (▒), plus monosodium dihydrogen phosphate (■), plus sodium hexametaphosphate (▥), plus pentasodium triphosphate (⁙), plus tetrasodium pyrophosphate (▤), and plus trisodium phosphate (▩). Samples labeled with different letters were considered to differ with statistical significance (*$P < 0.05$*) from one another with regard to the number of culturable* Campylobacter *cells present in each.*

influence of polyphosphate-containing marinades on *Campylobacter* survival in chicken exudates. However, exactly what the pH changes are doing physiologically to the organism will need to be addressed. Other research groups have performed microarray-based studies of *Campylobacter* with published results that focus on *C. jejuni* incubated in acidic pH conditions or in chicken exudate *(10, 13, 14)*. The microarray studies using chicken exudate without added marinades or polyphosphates investigated *Campylobacter* differential gene expression in exudates compared to gene expression in brain heart infusion laboratory media at refrigeration temperatures under normal atmospheric conditions *(10)*. The microarray studies investigating the effects of acidic conditions identified genes preferentially expressed by *C. jejuni* strains under various acid environments, but these experiments were performed at elevated temperatures (37°C) in a microaerobic environment *(13, 14)*. While the previous three studies will supply insights into the molecular mechanisms for the increased survival of *Campylobacter* in exudate, in order to more fully investigate the mechanisms, it is necessary to combine aspects of the previous studies with research utilizing conditions that adhere more closely to poultry processing and storage conditions. A microarray-based study comparing both *C. jejuni* and *C. coli* strains in exudate plus marinade versus exudate alone incubated at 4°C under a normal oxygen environment should give clear insights into what genes are being influenced by the presence of polyphosphate-containing marinades. The proposed microarray work in conjunction with research described in this article on the effect that pH changes mediated by polyphosphates have on the survival of *C. jejuni* and *C. coli* strains will present a more complete understanding of the food safety risk of polyphosphate usage in poultry.

## ACKNOWLEDGMENTS

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